


### REMARKS

The Specification, Abstract and claims have been amended to insert reference to SEQ ID NOS. found in the Sequence Listing. The Specification further has been amended to include the Sequence Listing, as required by the Notice to Comply (copy attached hereto). The Sequence Listing contains no new matter; therefore, its entry is requested.

RESPECTFULLY SUBMITTED,					
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Dated: 06 March 2001

**Attachments:** Sequence Listing  
Marked-Up Copies of Amendments  
*Statement Pursuant to 37 CFR 1.821(f)* with diskette

**Amended Specification Page 5: Version with markings to show changes made**

**SUMMARY OF THE INVENTION**

This invention relates to an isolated osteocalcin fragment derived from human urine, said fragment being characterized in that at least one of the glutamic acids in the position 17, 21 and 24 of the amino acid sequence

Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-Pro-Val-Pro-Tyr-Pro-Asp-Pro-Leu-  
17 21 24 30  
Glu-Pro-Arg-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Asp-Cys-Asp-Glu-Leu-  
Ala-Asp-His-Ile-Gly-Phe-Gln-Glu-Ala-Tyr-Arg-Arg-Phe-Tyr-Gly-Pro-

Val (SEQ ID NO:2)

is gamma-carboxylated.

**Amended Specification Page 7: Version with markings to show changes made**

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 A shows the nucleic acid (SEQ ID NO:1) and the amino acid (SEQ ID NO:2) sequences of the synthetic human osteocalcin insert (SEQ ID NO:3). Figure 1 B shows plasmid vector pGEX-3X (Pharmacia). The arrow indicates the SmaI-ligation site of the hOC insert;. The pfXa (protease factor Xa) cleavage site is located after the Ile-Glu-Gly-Arg -sequence (residues 1-4 of SEQ ID NO:4).

**Amended Claims: Version with markings to show changes made**

1 (Amended). An isolated osteocalcin fragment derived from human urine, said fragment characterized in that at least one of the glutamic acids in the position 17, 21 and 24 of the amino acid sequence

6      7

Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-Pro-Val-Pro-Tyr-Pro-Asp-Pro-Leu-

17                      21                      24                      30

Glu-Pro-Arg-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Asp-Cys-Asp-Glu-Leu-

Ala-Asp-His-Ile-Gly-Phe-Gln-Glu-Ala-Tyr-Arg-Arg-Phe-Tyr-Gly-Pro-

Val [(SEQ ID NO:1)] (SEQ ID NO:2)

is gamma-carboxylated.

7 (Amended) The immunoassay according to claim 6 characterized by employing monoclonal antibodies or recombinant antibody fragments specific to epitopes that have been identified on the gamma-carboxylated fragment of osteocalcin, wherein said fragment spans either

- i) from the amino acid in position 7 to the amino acid in position 30, or
- ii) from the amino acid in position 6 to the amino acid in position 30 of the amino acid sequence described in [SEQ ID NO:1] SEQ ID NO:2, and that all three glutamic acids in the positions 17, 21 and 24 of said sequence are gamma-carboxylated.

Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-Pro-Val-Pro-Tyr-Pro-Asp-Pro-Leu-  
17                    21                    24                    30  
Glu-Pro-Arg-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Asp-Cys-Asp-Glu-Leu-  
  
Ala-Asp-His-Ile-Gly-Phe-Gln-Glu-Ala-Tyr-Arg-Arg-Phe-Tyr-Gly-Pro-  
  
Val (SEQ ID NO:2) (I)